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Novel 23-Base-Pair Duplication Mutation in TSC1 Exon 15 in an Infant Presenting With Cardiac Rhabdomyomas

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Tuberous sclerosis (TSC) is a dominantly inherited disorder due to mutations at two gene loci, the TSC1 locus on chromosome 9q34 and the TSC2 locus on chromosome 16p13.3. The TSC2 and the TSC1 genes have now been cloned, enabling mutation analysis. We report results of mutation analysis in a sporadic case of TSC first identified in intra-uterine life on the basis of the presence of cardiac rhabdomyomas. Postnatally this infant was also found to have subependymal nodules on brain computed tomographic scan. Hypomelanotic macules were not detected neonatally or at 12 months of age. The specific TSC1 exon 15 mutation found in our patient has not previously been reported in cases of TSC. This mutation involves duplication of a 23-bp segment of DNA between two 9-bp repeated sequence elements within exon 15. These repeat elements are located between nucleotides 1892–1900 and between nucleotides 1915–1923 within the TSC1 gene sequence. It is likely that the presence of these two repeated elements predisposes to misalignment of DNA strands and unequal crossing over. The mechanism of origin of rhabdomyomas in TSC is reviewed. Loss of heterozygosity in the TSC gene regions has been reported in cardiac rhabdomyomas; however, these lesions are self-limiting in their growth. The basis for this self limiting proliferation is not clear. One interesting postulation is that cardiac rhabdomyomas may be due to delay or failure of apoptosis which occurs as part of the normal remodeling process in the heart. *Am. J. Med. Genet.* 84:346–349, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: cardiac rhabdomyoma; tuberous sclerosis; mutation analysis; TSC1; hamartin; 23 base pair duplication

INTRODUCTION

Tuberous sclerosis (TSC) is a dominantly inherited disorder due to mutations at two gene loci, the TSC1 locus at 9q34 and the TSC2 locus at 16p13.3. Over 60% of cases of TSC are thought to be due to new mutations since an affected child has unaffected parents [Gomez, 1988]. We report on a sporadic case of TSC first identified in intra-uterine life on the basis of the presence of cardiac rhabdomyomas, in whom a novel TSC1 mutation occurred, due to the presence of a 23-base pair (bp) duplication in TSC1 exon 15. We initiated TSC1 gene analysis with examination of exon 15 since this exon is reported to have a higher frequency of mutation than any other TSC1 exon [van Slegtenhorst et al., 1997; Jones et al., 1997].

CLINICAL REPORT

The patient reported on here was found to have a left ventricular cardiac mass on routine fetal ultrasonography during the third trimester of pregnancy. Postnatal echocardiography demonstrated a small mass in the left ventricular apex and a larger mass in the right ventricular apex. On the basis of clinical examination cardiac function was judged uncompromised. No arrhythmias were detected. Electrocardiogram was normal. Computed tomographic (CT) scan of the brain revealed a 5-mm subependymal nodule in the left ventricle near the foramen of Munro. Magnetic resonance imaging (MRI) of the brain detected abnormal signal in same region in which the CT scan had detected a subependymal nodule. On MRI the presence of areas of increased signal of less than 3 mm in the lateral ventricles was noted and these areas were thought to represent additional tiny subependymal nodules. No cortical tubers were noted. Renal sonogram revealed no abnormalities. No hypomelanotic macules were detected on Wood's lamp examination of the skin. Given the fact that two hamartomas were found in this pa-

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tient (cardiac rhabdomyomas and subependymal nodules) a presumptive diagnosis of tuberous sclerosis was made [Gomez, 1988]. Follow-up examination at the age of one year showed normal growth and development. No seizures were reported. Wood's lamp examination of the skin failed to reveal any hypomelanotic macules. Echocardiogram demonstrated that the cardiac masses had not undergone size change. Cardiac function and rhythm were normal.

This infant was the only child of his parents. There was no family history of cardiac problems, seizures, developmental delay, or renal problems. Wood's lamp examination of the parents failed to reveal hypomelanotic macules and there were no other dermatologic abnormalities. Cranial CT and renal ultrasound studies were carried out on the parents. No abnormalities were detected.

METHODS

Blood samples were obtained from the patient and his parents for DNA extraction and for mutation analysis of the TSC genes. Mutation analysis was initiated using primers for TSC1 exon 15 and polymerase chain reaction (PCR). The specific exon 15 primers used are described in Table I. PCR fragments were electrophoresed on acrylamide gels to examine the size of the products generated. PCR products were also examined by heteroduplex analysis [Ganguly et al., 1993]. Following the detection of different size fragments in the patient, individual fragments were eluted from acrylamide gels using the method described by Maniatis et al. [1989]. Individual fragments were then subcloned into pUC vectors for analysis and DNA sequencing. DNA sequencing was carried out using the ABI Prism Tm dRhodamine Terminator cycle sequencing. The products of each sequencing reaction were precipitated and analyzed on an ABI sequencer.

RESULTS

PCR was initially carried out using primers (H21 and H22) to amplify the complete exon 15 of TSC1 (Table I). Polyacrylamide gel electrophoresis showed that there was a different pattern of PCR products present in the patient than in the parents. Comparison of PCR bands with molecular weight markers indicated that in the parents a single 559 nucleotide fragment was present, while in the patient there were two closely migrating bands of 559 and 582 nucleotides and an additional fragment of 1164 nucleotides. PCR frag-

ments from the patient were individually purified and subcloned into pUC vectors using blunt end ligation into the SMA1 site of pUC. We demonstrated that the 1164-bp fragment arose during PCR from the 582-bp band by purifying the 582-bp band and demonstrating that a second round of PCR of this band led to generation of the 582-bp band plus a 1164-bp band (Fig. 1). A second round of PCR of the 559-bp band from controls and from the patient did not lead to the generation of the 1164-bp band. We then carried out PCR using primers which were located within TSC exon 15 as described by Jones et al. [1997]. These primer sets each amplified approximately one-third of TSC exon 15. We demonstrated differences between the patient and parents with primer sets 15/1 and with primer set 15/2. No difference was demonstrated in PCR products derived from parents and child using primer set 15/3 which amplified the last third of exon 15 (closest to the C terminal portion of the gene). Based on these results we concluded that the sequence alteration in the patient occurred 5' to the region amplified by primer set 15/3 (i.e., 5' to nucleotide 2064). DNA sequence analysis revealed that there was a 23 nucleotide duplicated segment present in the patient. Examination of the hamartin sequence demonstrated that there is a 9-bp repeat, GCCCTGCGG, located between nucleotides 1892–1900 and also between nucleotides 1915–1923. The duplicated segment present in the patient comprises an additional copy of the region between the two repeated elements and an additional copy of one of the repeated elements (Fig. 2).

DISCUSSION

We have demonstrated the occurrence of a mutation in TSC1 exon 15 in an infant who presented with cardiac rhabdomyoma detected in the third trimester of pregnancy. Postnatally this infant was also found to have subependymal nodules on brain CT scan. Based on the absence of clinical findings in the parents and the absence of mutation in DNA prepared from their peripheral blood, it seems most likely that this infant represents a sporadic case (new mutation) of TSC. However, it is important to note that our studies cannot rule out the possibility of gonadal mosaicism in one parent.

There are a number of interesting aspects in this case. This specific mutation is unique and has not been described previously in the TSC1 gene. This infant apparently represents a sporadic case of TSC and studies

TABLE I. DNA Sequence of Four Different Primer Sets Used to Amplify TSC1 Exon 15

| Exon | Primer designation | Sequence | Product size | Reference |
|------|--------------------|-----------------------------|--------------|--------------------------------|
| 15 | H21 | 5' GAGAGTGCCCCAGTCCCTTAC 3' | 559 bp | van Slegtenhorst et al. [1997] |
| | H22 | 5' CCAGGTGGAATACCGACTGC 3' | | |
| 15-1 | F | 5' GAATACCGACTGCCATTTCT 3' | 303 bp | Jones et al. [1997] |
| | R | 5' AGGGCTTTCATCAGCACTG 3' | | |
| 15-2 | F | 5' GCAAGCCTTFACTCCCATAG 3' | 276 bp | Jones et al. [1997] |
| | R | 5' GGCACACCATCTTCCTCTG 3' | | |
| 15-3 | F | 5' CAGCCATCATTTTGTCATC 3' | 256 bp | Jones et al. [1997] |
| | R | 5' AGGTGGGAGTGTGAAGAATG 3' | | |

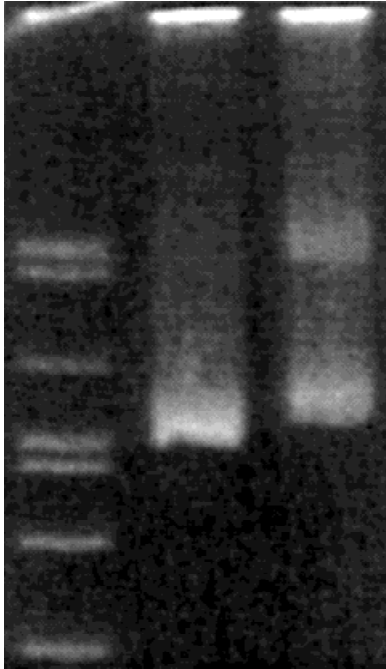


Fig. 1. PAGE of PCR fragments derived from patient using primers TSC1 exon 15 H21/H22. The 559- and 582-bp fragments were individually purified and subjected to a second round of PCR. Left to right: Lane 1, DNA size markers (from top to bottom) 1114, 900, 692, 501, 489, 404, and 320 bps; Lane 2, PCR of the 559-bp fragment yielded only one product; Lane 3, PCR of the 582-bp fragment yielded two products, 582 and 1164 bps.

to date indicate that TSC1 mutations occur less frequently in sporadic cases of TSC than in familial cases. There are also a number of unusual clinical aspects in this infant. Cardiac rhabdomyomas were detected on routine ultrasonography in the third trimester of pregnancy. There was no evidence of regression of the cardiac rhabdomyomas after the first year of life. Despite the relatively large size of the rhabdomyomas they did not compromise cardiac function and did not cause arrhythmias. Hypomelanotic macules were not detected in the neonatal period or at one year of age. Other manifestations of TSC including angiofibroma, shagreen patches, renal cysts, and renal angiomyolipomas were not detected neonatally or at one year of age.

It is estimated that hypomelanotic macules develop in more than 90% of cases with TSC [Gomez, 1988]. Jozwiak et al. [1994] noted that depigmented lesions were found neonatally in 44 of the 51 (86%) cases of

TSC reported by them. Although in many cases hypomelanotic macules can be detected by Wood's lamp examination shortly after birth, there are reports of children with TSC in whom hypomelanotic macules were not detected at birth or in the first few months of life. Wallace et al. [1990] described five infants in whom cardiac tumors were identified on ultrasonography in fetal or early postnatal life. No hypomelanotic macules were detected at birth in any of these infants although in four of the five infants they developed later, before the age of 2 years. Seizures developed in four of the five infants before the age of 2 years. In one infant Wallace et al. [1990] noted that multiple cardiac tumors were diagnosed shortly after birth. Examination of the skin at birth and at 2 months and 5 months failed to show signs of TSC. A depigmented macule and a depigmented streak of hair appeared by 12 months and during the course of the following year additional depigmented patches appeared. A second case in the series described by Wallace et al. [1990] was noted to have a normal heart on fetal ultrasound at 34 weeks; however, at 35 weeks an echodense area was detected in the right ventricle. Neonatally this infant had arrhythmias and numerous cardiac rhabdomyomas were detected. No hypomelanotic macules were detected in this infant until the age of 2 years. Although CT scan of the brain revealed no lesions, the infant developed atypical absence seizures at the age of 2 years. In the third infant in the series of Wallace et al. [1990] depigmented lesions developed at 14 months. In the fourth infant in this series depigmented lesions developed at 5 months. In the fifth infant in their series Wallace et al. [1990] diagnosed a rhabdomyoma causing cardiac outflow obstruction. At 4 months seizures developed in this infant and brain CT scan revealed multiple periventricular calcifications. At the age of 18 months no hypomelanotic macules were detected. Wallace et al. [1990] concluded that in infants with cardiac rhabdomyomas the diagnosis of TSC must not be discounted because of a normal skin examination and normal brain CT scan findings.

The precise mechanism of origin of the hypomelanotic macules in TSC and the timing of their appearance are poorly understood. Fitzpatrick [1991] described histologic analysis of the hypomelanotic macules. They noted that melanocytes were reduced in number, and melanosomes were present but the melanin content was markedly reduced in the lesions relative to normal skin.

Lie [1991] reported that cardiac rhabdomyomas in patients with TSC may originate in any of the four chambers of the heart but that they were more common in the ventricles than in the atria and that they occurred more frequently on the left side of the heart than the right side. Bosi et al. [1996] carried out a retrospective study of cardiac rhabdomyomas in 33 children. They reported that 30 of the 33 cases were associated with TSC. A solitary tumor was detected in 10 patients. In 23 patients the tumor number ranged between two and five. The youngest patient with evidence of total regression of tumors was 7 years old. The youngest patient in whom partial regression of cardiac rhabdomyomas was noted was 6 weeks old. Jozwiak et

Normal Sequence

GCCCTGCCGGCAGTGCTGATGAAAGCCCTGCCGG GA
 1892 1900 1915 1923

Patient Sequence

GCCCTGCCGGCAGTGCTGATGAAAGCCCTGCCGGAGTGCTGATGAAAGCCCTGCCGG GA
 1892 1900 1915 1923

Fig. 2. Comparison of partial TSC1 sequence in patient with normal sequence. Note that two copies of the 9 nucleotide duplicated segment (GCCCTGCCGG) are present in the normal sequence at positions 1892 to 1900 and at positions 1915 to 1923. Note that in the patient three copies of the 9 nucleotide sequence are present. The 23-bp duplicated segment includes the 9 nucleotide repeat and 14 nucleotides corresponding to sequence between the two repeats.

al. [1994] reported results of echocardiography on 29 children with TSC who were followed in a child neurology clinic. Cardiac rhabdomyomas were detected in 10 of these children. They noted that cardiac rhabdomyomas were more common on the left side of the heart than the right and that they were more common in the intraventricular septum than in the ventricular wall. Results of mutation analysis indicate that cardiac rhabdomyomas are a feature of TSC1 and TSC2. Van Slegtenhorst et al. [1998] demonstrated TSC1 mutations in 6 out of 28 cases with cardiac rhabdomyomas. Au et al. [1998] described TSC2 mutations in 22 cases of familial or sporadic TSC. In six of these cases cardiac rhabdomyomas were present. A review of the loss of heterozygosity in different TSC lesions shows that in four of nine cases of cardiac rhabdomyoma LOH was detected in the TSC2 gene region on chromosome 16p13.3 [Green et al., 1994a; Henske et al., 1996; Sepp et al., 1996].

It is interesting to note that although loss of heterozygosity in the TSC gene region has been noted in cardiac rhabdomyomas, these lesions are self-limiting in their growth. The basis for this self-limiting proliferation is not clear. One very interesting postulation is that the presence of the cardiac rhabdomyomas may be due to delayed or failed apoptosis which occurs as part of the normal remodeling process in the heart. Satge and De Geeter [1992] noted that apoptosis is observed in tumors which show signs of involution. Medioni et al. [1994] proposed that cardiac rhabdomyomas should be considered as developmental vestiges rather than true hamartomas. They proposed that the rhabdomyomas represent vestigial structures resulting from a defect in differentiation or a defect in regression of primitive myocardial cells. It is also possible that other factors, such as the influence of positive or negative growth factors, may determine the growth or regression of cardiac rhabdomyomas.

Exon 15 represents the TSC1 exon with the highest frequency of mutations [van Slegtenhorst et al., 1997, 1998]. The specific TSC1 exon 15 mutation found in our patient has not previously been reported in cases of TSC. This mutation involves duplication of a 23-bp segment of sequence between two 9-bp repeated sequence elements within exon 15. These repeat elements are located between nucleotides 1892–1900 and between nucleotides 1915–1923 within hamartin. It is likely that the presence of these two repeated elements predisposes to misalignment of DNA strands and unequal crossing over. It is of particular interest to note that van Slegtenhorst et al. [1998] described a 23-bp deletion originating at bp 1892 in TSC1 exon 15. This deletion would include one of the 9-bp repeat elements and the region between the two repeated elements. Their finding further strengthens our postulation that the presence of the repeated elements within exon 15 predisposes to mispairing of strands and unequal crossing over.

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